Death, Cardiac Dysfunction, and Arrhythmias Are Increased by Calmodulin Kinase II in Calcineurin Cardiomyopathy

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Background—Activation of cellular Ca2+/H11001 signaling molecules appears to be a fundamental step in the progression of cardiomyopathy and arrhythmias. Myocardial overexpression of the constitutively active Ca2+-dependent phosphatase calcineurin (CAN) causes severe cardiomyopathy marked by left ventricular (LV) dysfunction, arrhythmias, and increased mortality rate, but CAN antagonist drugs primarily reduce hypertrophy without improving LV function or risk of death.

Methods and Results—We found that activity and expression of a second Ca2+-activated signaling molecule, calmodulin kinase II (CaMKII), were increased in hearts from CAN transgenic mice and that CaMKII-inhibitory drugs improved LV function and suppressed arrhythmias. We devised a genetic approach to “clamp” CaMKII activity in CAN mice to control levels by interbreeding CAN transgenic mice with mice expressing a specific CaMKII inhibitor in cardiomyocytes. We developed transgenic control mice by interbreeding CAN transgenic mice with mice expressing an inactive version of the CaMKII-inhibitory peptide. CAN mice with CaMKII inhibition had reduced risk of death and increased LV and ventricular myocyte function and were less susceptible to arrhythmias. CaMKII inhibition did not reduce transgenic overexpression of CAN or expression of endogenous CaMKII protein or significantly reduce most measures of cardiac hypertrophy.

Conclusions—CaMKII is a downstream signal in CAN cardiomyopathy, and increased CaMKII activity contributes to cardiac dysfunction, arrhythmia susceptibility, and longevity during CAN overexpression. (Circulation. 2006;114:1352-1359.)

Key Words: arrhythmia ■ calcium ■ cardiomyopathy ■ signal transduction

Multiple cellular signals are altered in structural heart disease, so it is often unclear how these signals individually contribute to disease phenotypes. The multifunctional Ca2+/H11001 and calmodulin-dependent protein kinase II (CaMKII) has recently emerged as a mediator of cardiomyopathic signaling in patients with end-stage heart failure from a variety of causes.1,2 Increased CaMKII activity is linked to arrhythmias,3,4 sudden death,5 and mechanical dysfunction,5 the cardiomyopathic phenotypes most closely associated with increased mortality rate in patients with structural heart disease.6 These findings led us to hypothesize that CaMKII links mechanical and electrical phenotypes in cardiomyopathy.7 Myocardial CaMKII inhibition can reduce cardiac dysfunction after excessive catecholamines or myocardial infarction,8 but there is no evidence that CaMKII inhibition can reduce arrhythmias or improve mortality in severe cardiomyopathy, a condition with few effective pharmacological therapies.6

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Transgenic (TG) cardiac overexpression of a constitutively active form of calcineurin (CAN) causes hypertrophy, severe mechanical dysfunction, arrhythmias, and premature death in mice.9,10 Increases in CAN activity and/or expression also are reported in other animal models11 and in patients12 with structural heart disease. Pharmacological CAN inhibition reduces cardiac hypertrophy in CAN-overexpressing mice9...
but surprisingly does not reduce mortality or improve left ventricular (LV) function, which suggests that other signals may persist even after CAN inhibition that are critical for increased mortality in CAN cardiomyopathy. CaMKII becomes autonomous after initial activation and thus could be a persistent stimulus for LV dysfunction and arrhythmias even after CAN inhibition. We used pharmacological and genetic approaches to inhibit CaMKII in mice with TG cardiac CAN overexpression to test the hypothesis that CaMKII activity significantly contributes to mechanical dysfunction, arrhythmia susceptibility, and mortality in the CAN-overexpression model of severe cardiomyopathy. Here, we show that mice with cardiac CAN overexpression have elevated CaMKII activity and expression and that CaMKII inhibition decreases mortality, suppresses cardiac arrhythmias, and improves mechanical function. On the other hand, CaMKII inhibition did not reduce most measures of cardiac hypertrophy. These findings show that CaMKII is an important but previously unrecognized signal in CAN cardiomyopathy and suggest that increased CaMKII activity increases arrhythmia susceptibility, increases mechanical dysfunction, and contributes to increased mortality.

Methods

CAN AC3-I and AC3-C TG Mice

CAN TG mice were engineered as previously described. AC3-I and AC3-C mice also were previously described. Briefly, the AC3-I and AC3-C mice were generated by synthesis of a minigene based on the peptide sequence of the CaMKII-inhibitory peptide AC3-I (KKAL-HQEAVDCL) or the inactive control peptide AC3-C (KKAL-HAQERVDC). CAN TG mice were injected daily with the CaMKII-inhibitory agent KN-93 (10 mol/kg IP) or the inactive control peptide AC3-C (KKAL-H9262). CAN TG mice were engineered as previously described. AC3-I and AC3-C mice also were previously described. Briefly, the AC3-I and AC3-C mice were generated by synthesis of a minigene based on the peptide sequence of the CaMKII-inhibitory peptide AC3-I (KKAL-HQEAVDCL) or the inactive control peptide AC3-C (KKAL-HAQERVDC). CAN TG mice were injected daily with the CaMKII-inhibitory agent KN-93 (10 mol/kg IP) or the inactive control agent KN-92 (30 mol/kg IP) for some studies. The University of Iowa and Vanderbilt University Divisions of Animal Care approved all procedures.

CaMKII Activity Assays

CaMKII activity of ventricular homogenates was measured against a synthetic substrate (syntide 2) as described elsewhere.

Mechanical Studies in Isolated Ventricular Myocytes

Cellular shortening and relaxation were measured in paced ventricular myocytes at ambient temperature (23°C to 25°C) as previously described (Ionoptix). Ventricular myocyte cross-sectional area was measured from middle transverse LV sections (7 μm) showing cells with a centrally located nucleus using ImagePro Plus software. Alternate sections were stained with Masson’s trichrome, and the area of fibrosis was quantified with ImagePro Plus software.8 Alternate sections

ECG Telemetry and Arrhythmia Scoring In Vivo

Surgical implantation of an ECG telemeter (Data Sciences International, St Paul, Minn) and arrhythmia scoring and quantification were performed as previously described. Details are provided in the online Data Supplement.

Arrhythmia Scoring in Langendorff-Perfused Hearts

Hearts were removed from mice anesthetized with avertin (20 μg/10 g body weight, 2.5% solution: 10 g tribromoethanol alcohol, 10 mL tert-amylalcohol, stored at 4°C as a stock solution) with 1 mg/mL heparin (Sigma, St Louis, Mo; 171 USP unit/mg, 20 μL/kg). Excised hearts were mounted on a modified Langendorff apparatus (HSE-HA perfusion systems, Harvard Apparatus, Holliston, Mass) for retrograde aortic perfusion at a constant pressure of 80 mm Hg with oxygenated (95% O2, 5% CO2) Krebs-Henseleit solution consisting of (in mmol/L) 25 NaHCO3, 118 NaCl, 4.7 KCl, 1.2 CaSO4, 1.2 NaH2PO4, 2.5 CaCl2, 0.5 Na-EDTA, and 15 glucose, with pH equilibrated to 7.4. The perfused heart was immersed in the water-jacked bath and was maintained at 36°C.

After the heart was allowed to stabilize for 10 minutes, baseline ECG recordings were made over 5 minutes after Ag+-AgCl electrodes were positioned around the heart in an approximate Einthoven configuration as described elsewhere. Pacing was performed with a paired unipolar electrode configuration (Medtronic model 2356 custom programmable stimulator, Medtronic Inc, Minneapolis, Minn; 1.0-ms pulse width) at twice the capture threshold using 50-, 80-, 100-, and 150-ms drive cycles. Single and double extrastimuli were added to the drive cycles and decreased in 1- to 10-ms increments. After completion of the baseline (5 minutes) and pacing (5 minutes) measurements, hearts were perfused with 1 μmol/L isoproterenol (5 minutes). The arrhythmia score was measured as previously described (see the experimental Methods section of the online Data Supplement for basal, pacing, and isoproterenol conditions). In vitro arrhythmias were reported as arrhythmia burden products for the 5-minute epoch or during periods immediately after pacing.

Real-Time PCR

Total RNA from TG mice and control littersmates was prepared from frozen heart tissues with RNAwiz reagent (Ambion). Reverse transcription was performed using the Taqman reverse-transcription kit (Applied Biosystems) with 2 μg total RNA and 2.5 μmol/L random hexamer primers at 48°C for 30 minutes. The polymerase chain reaction (PCR) conditions are detailed in the expanded Methods section in the online Data Supplement.

Histological Measurements

Ventricular myocyte cross-sectional area was measured from middle transverse LV sections (7 μm) showing cells with a centrally located nucleus using ImagePro Plus software. Alternate sections were stained with Masson’s trichrome, and the area of fibrosis was quantified with Image Pro plus software. The investigator was blinded to the genetic identity of the tissue sections.

TUNEL Staining

TUNEL assays were performed with the In Situ Cell Death Detection Kit (Roche), according to the manufacturer’s instruction (see Methods in online Data Supplement).

Statistical Analysis

Data analysis was performed with ANOVA, repeated-measures ANOVA, Student t test, the Mann-Whitney rank-sum test, or a log-rank test, as appropriate, with SigmaStat software. Post-hoc comparisons by ANOVA were analyzed using Bonferroni’s correction. Values are mean±SEM. The null hypothesis was rejected for values of P<0.05.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written.
Results

Increased CaMKII Activity in CAN Cardiomyopathy

CaMKII activity was significantly increased in CAN TG hearts compared with WT littermate controls (Figure 1A), potentially consistent with the concept that increased CaMKII activity contributed to cardiomyopathic phenotypes in CAN TG mice. CaMKII activity was significantly reduced by the addition of AC3-I peptide (10 μmol/L) to cardiac homogenates from WT and CAN mice compared with homogenates without added peptide (P<0.001). There was a trend (P=0.07) toward more residual CaMKII activity in CAN homogenates compared with WT homogenates, in line with the greater basal CaMKII activity in CAN compared with WT hearts.

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CaMKII inhibition reduces arrhythmias and improves LV function in CAN cardiomyopathy. A, CaMKII activity is significantly increased (tP<0.001) in cardiac homogenates from CAN mice (n=5) compared with WT littermate controls (n=4). The CaMKII-inhibitory peptide AC3-I (10 μmol/L) significantly (P<0.001) reduced CaMKII activity in CAN and WT hearts. B, Daily injection with the CaMKII-inhibitory drug KN-93 (●) significantly improved LV fractional shortening in CAN TG mice at 59 (*P=0.02) and 73 (tP=0.04) days compared with CAN mice injected with the control drug KN-92 (○) but did not significantly change LV fractional shortening compared with baseline (P=0.1). The injections began after the baseline measurements marked by arrowheads. LV fractional shortening remained significantly worse in CAN mice injected with KN-93 or KN-92 compared with WT littermate controls (○) (tP<0.001). Each point represents data from 4 to 8 mice. C, Ventricular tachycardia recorded with ECG telemetry in a CAN mouse. The horizontal scale bar indicates 200 ms. D, Summary data for the arrhythmia burden product in CAN and WT littermate controls under basal conditions or after drug treatment. The arrhythmia burden product was significantly greater in CAN than in WT mice under basal conditions (tP=0.002), after isoproterenol (iso; tP=0.002), and after the inactive control drug KN-92 (tP=0.015). Treatment with the CaMKII inhibitor KN-93 or the calmodulin antagonist W-7 both significantly suppressed arrhythmias in CAN mice; the control drug KN-92 was ineffective. In contrast, none of these agents affected arrhythmia scores in WT control mice. These findings suggested that cardiac dysfunction and arrhythmias in CAN mice are related to increased CaMKII activity and showed that systemic application of a CaMKII-inhibitory drug can suppress these phenotypes.

CaMKII Inhibition Improves Cardiac Function and Suppresses Arrhythmias

To test whether reduced LV function and increased arrhythmias in CAN mice are related to the increase in CaMKII activity (Figure 1A), we injected CAN TG mice with the CaMKII-inhibitory drug KN-93 or the control drug KN-92 daily for 21 days. KN-93 treatment significantly improved LV function compared with KN-92 (Figure 1B). CAN mice had frequent arrhythmias, whereas WT mice were not prone to arrhythmias at baseline or after provocation with isoproterenol (Figure 1, C and D). KN-93 and the calmodulin-inhibitory drug W-7 both significantly suppressed arrhythmias in CAN mice; the control drug KN-92 was ineffective. In contrast, none of these agents affected arrhythmia scores in WT control mice. These findings suggested that cardiac dysfunction and arrhythmias in CAN mice are related to increased CaMKII activity and suggested that systemic application of a CaMKII-inhibitory drug can suppress these phenotypes.

CAN Overexpression Increases CaMKII mRNA

CaMKII upregulation occurs in structural heart disease from a wide range of causes, raising the question of whether the increased CaMKII activity seen in CAN-overexpressing mice is a downstream consequence of CAN or is triggered by other signals arising in the cardiomyopathic milieu. To address this question, we infected isolated neonatal rat cardiomyocytes with adenovirus encoding GFP and constitutively active CAN (right). The same viewing field is shown in each of the columns, with incandescent light (ICD; top), fluorescent light to excite GFP (middle), and DAPI staining to show nuclei (bottom). The scale bars equal 20 μm throughout. B, Immunoblots from cellular homogenates for CAN (top) and β-actin (bottom) show overexpression of the truncated, constitutively active form of CAN (arrow) in cells infected with GFP-CAN-encoding adenovirus. C, Real-time PCR measurements of CaMKIIβ show a significant increase in mRNA 24 hours after infection with CAN- and GFP-encoding adenovirus (tP=0.04). Data are from 3 separate experiments.

**Figure 1.** CaMKII inhibition reduces arrhythmias and improves LV function in CAN cardiomyopathy. A, CaMKII activity is significantly increased (tP<0.001) in cardiac homogenates from CAN mice (n=5) compared with WT littermate controls (n=4). The CaMKII-inhibitory peptide AC3-I (10 μmol/L) significantly (P<0.001) reduced CaMKII activity in CAN and WT hearts. B, Daily injection with the CaMKII-inhibitory drug KN-93 (●) significantly improved LV fractional shortening in CAN TG mice at 59 (*P=0.02) and 73 (tP=0.04) days compared with CAN mice injected with the control drug KN-92 (○) but did not significantly change LV fractional shortening compared with baseline (P=0.1). The injections began after the baseline measurements marked by arrowheads. LV fractional shortening remained significantly worse in CAN mice injected with KN-93 or KN-92 compared with WT littermate controls (○) (tP<0.001). Each point represents data from 4 to 8 mice. C, Ventricular tachycardia recorded with ECG telemetry in a CAN mouse. The horizontal scale bar indicates 200 ms. D, Summary data for the arrhythmia burden product in CAN and WT littermate controls under basal conditions or after drug treatment. The arrhythmia burden product was significantly greater in CAN than in WT mice under basal conditions (tP=0.002), after isoproterenol (iso; tP=0.002), and after the inactive control drug KN-92 (tP=0.015). Treatment with the CaMKII inhibitor KN-93 or the calmodulin antagonist W-7 both significantly suppressed arrhythmias in CAN mice; the control drug KN-92 was ineffective. In contrast, none of these agents affected arrhythmia scores in WT control mice. These findings suggested that cardiac dysfunction and arrhythmias in CAN mice are related to increased CaMKII activity and showed that systemic application of a CaMKII-inhibitory drug can suppress these phenotypes.

**Figure 2.** CAN upregulates CaMKIIβ in neonatal cardiomyocytes. A, Micrographs showing neonatal rat cardiomyocytes treated with empty adenovirus (left) and adenovirus encoding GFP and constitutively active CAN (right). The same viewing field is shown in each of the columns, with incandescent light (ICD; top), fluorescent light to excite GFP (middle), and DAPI staining to show nuclei (bottom). The scale bars equal 20 μm throughout. B, Immunoblots from cellular homogenates for CAN (top) and β-actin (bottom) show overexpression of the truncated, constitutively active form of CAN (arrow) in cells infected with GFP-CAN-encoding adenovirus. C, Real-time PCR measurements of CaMKIIβ show a significant increase in mRNA 24 hours after infection with CAN- and GFP-encoding adenovirus (tP=0.04). Data are from 3 separate experiments.
CaMKII Inhibition Improves Mortality in CAN TG Mice

Myocardial CAN overexpression begins near birth in CAN TG mice because of the developmentally programmed switching to α-myosin heavy chain (MHC) expression. Pharmacological CaMKII inhibition is impractical over a similar time course, and currently available CaMKII antagonist drugs, including KN-93 and W-7, are notorious for acting as CaMKII-independent ion channel antagonists. To overcome these obstacles, we interbred CAN mice with recently developed mice with genetic myocardial CaMKII inhibition resulting from α-MHC promoter-driven expression of a highly specific CaMKII-inhibitory peptide, AC3-I (I indicates inhibitor). AC3-I is modeled after the autoinhibitory region of the CaMKII regulatory domain (Figure 3A). AC3-C (C indicates control) is a scrambled version of AC3-I without CaMKII-inhibitory activity. We did not detect overt differences in mechanical function between AC3-I and AC3-C mice at baseline. AC3-C TG mice were used to test for a potential mortality benefit from CaMKII inhibition resulting from α-MHC promoter-driven expression of a highly specific CaMKII-inhibitory peptide, AC3-I (I indicates inhibitor). AC3-I is modeled after the autoinhibitory region of the CaMKII regulatory domain (Figure 3A). AC3-C (C indicates control) is a scrambled version of AC3-I without CaMKII-inhibitory activity. We did not detect overt differences in mechanical function between AC3-I and AC3-C mice at baseline. AC3-C TG mice were used to generate a new line of control animals by interbreeding with CAN TG mice. Both AC3-I and AC3-C were expressed as fusion proteins with enhanced green fluorescent protein (EGFP), and enhanced GFP expression is apparent under fluorescent light in hearts from both CAN mice interbred with AC3-I and AC3-C TG mice (Figure 3, B and C).

CaMKII activity was reduced to WT levels in CAN×AC3-I mice, whereas cardiac CaMKII activity in CAN×AC3-C mice remained unchanged compared with CAN TG hearts (Figure 3D). We considered the possibility that TG CAN expression might be reduced in the interbred mice as a result of competition for α-MHC promoter activity. However, TG CAN expression was not different between CAN, CAN×AC3-I, and CAN×AC3-C hearts (Figure 3E), showing that the interbreeding approach was successful in selectively targeting myocardial CaMKII activity. Endogenous CaMKII was increased in CAN TG hearts, and CaMKII expression was not affected by AC3-I or AC3-C (Figure 3E). These findings support a model in which increased CaMKII expression and activity are a downstream consequence of TG CAN overexpression. Myocardial CaMKII overexpression increases mortality, so we interbred 30 CAN×AC3-I and 30 CAN×AC3-C mice to test for a potential mortality benefit from CaMKII inhibition. CAN×AC3-I mice did exhibit a significant mortality advantage compared with CAN×AC3-C mice (Figure 3F). This finding supports the hypothesis that CaMKII is important for determining longevity in CAN cardiomyopathy and is consistent with reports of premature death in mice with cardiomyopathy resulting from myocardial CaMKII overexpression.

Contraction Is Improved by CaMKII Inhibition

We measured LV fractional shortening in unanesthetized mice from 6 to 12 weeks of age (Figure 4, A and B). CAN (P=0.028) and CAN×AC3-C (P=0.002) mice had progressive deterioration of LV fractional shortening, but mechanical function did not significantly decline in CAN×AC3-I mice. There were no significant differences in LV septal thickness (Figure 4C) or LV diameter in diastole (Figure 4D). The improvement in LV fractional shortening was due primarily to improved LV systolic function.

Figure 3. Genetic cardiac CaMKII inhibition in CAN mice. A, Schematic of CaMKII showing the amino acid sequences for the inhibitory peptide AC3-I, which mimics key residues in the CaMKII regulatory domain, and the scrambled inactive control peptide AC3-C. The discrepant amino acids between AC3-I and AC3-C are in bold, and the arrow indicates the Ala that is substituted in AC3-I for Thr287 in CaMKII to eliminate potential inactivating effects of Thr phosphorylation. B, Hearts isolated from WT, CAN, CAN×AC3-I, and CAN×AC3-C interbred mice under incandescent light. The calibration bar indicates 5 mm. C, Fluorescent light reveals enhanced GFP expression in the AC3-I and AC3-C interbred mice. D, CAN×AC3-I hearts (n=5) had significantly less (†P<0.001) total CaMKII activity compared with CAN×AC3-C hearts (n=4). Bars indicate that CaMKII activity was not different between WT and CAN×AC3-I hearts or between CAN and CAN×AC3-C hearts. E, Immunoblots for CaMKII (top row), the TG catalytic subunit of CAN (middle row), and GAPDH (bottom row). Each lane was loaded with homogenate from separate hearts, and genetic identities are indicated on the top. F, Kaplan-Meier analysis of CAN×AC3-I (n=30) and CAN×AC3-C (n=30) interbred mice shows a significant difference in longevity (P<0.05).
function in CAN mice with CaMKII inhibition (Figure 4E). There was no significant decline in LV systolic diameter in CAN×AC3-I mice, but other TG mice did decline significantly: CAN (P = 0.026) and CAN×AC3-C (P = 0.029) from week 6 to 12. Studies in isolated ventricular myocytes from CAN×AC3-I verified improved fractional shortening (Figure 5, A and B) and relaxation (Figure 5C) compared with CAN×AC3-C hearts. There were no differences in ryanodine receptors (RyRs), sarcoplasmic endoplasmic reticulum Ca²⁺ ATPase (SERCA2a), or phospholamban (PLN) between CAN×AC3-I and CAN×AC3-C hearts (Figure 5D, through G), but both lines of interbred mice had lower RyRs than CAN or WT mice (Figure 5E). There was no detectable PLN phosphorylation at the CaMKII site (Thr17) in CAN×AC3-I hearts, whereas CaMKII-dependent PLN phosphorylation was detected in WT, CAN, and CAN×AC3-C hearts (Figure 5, D and I). Taken together, these findings support results with pharmacological CaMKII inhibition (Figure 1) and are consistent with the hypothesis that increased CaMKII activity is important for reduced LV function in CAN cardiomyopathy.
CAN×AC3-C mice, but all TG mice showed significant increases in heart weight compared with WT controls \( (P<0.001) \). CAN×AC3-I hearts did exhibit a modest \( (\approx 8\%) \) but significant reduction in cross-sectional cardiomyocyte area compared with CAN×AC3-C or CAN TG mice (Figure 6, C and D). All of the TG strains had significantly increased cell area compared with WT controls \( (P<0.001) \). There was no reduction in the hypertrophy marker gene brain natriuretic peptide (BNP) in CAN×AC3-I compared with CAN×AC3-C or CAN hearts (Figure 6E). Cardiac hypertrophy is marked by a relative preference for the \( \beta \)-MHC isofrom that mimics the condition of neonatal heart.\(^24\) \( \beta \)-MHC message was significantly greater in CAN×AC3-C and CAN×AC3-I than in WT hearts (Figure 6F). Overall, these findings show that CaMKII inhibition does not consistently reduce measures of cardiac hypertrophy in mice with overexpression of a constitutively active form of CAN.

CaMKII Inhibition Does Not Affect Apoptosis in CAN-Expressing Mice
CaMKII can enhance apoptosis in cardiomyocytes,\(^23\) suggesting that CaMKII inhibition could improve LV fractional shortening in CAN×AC3-I mice at least in part by reducing cell death. We did not detect evidence of significant apoptosis or fibrosis in any of the mice (see online Data Supplement), suggesting that increased CaMKII-dependent cell death is not a significant determinant of mechanical dysfunction in CAN cardiomyopathy.

CaMKII Inhibition Reduced Stimulated Arrhythmias
We calculated arrhythmia burden products in CAN×AC3-I and CAN×AC3-C mice implanted with ECG telemeters after saline or isoproterenol injection. Surprisingly, the arrhythmia burden products were lower at baseline and after isoproterenol in CAN×AC3-I (baseline, 3.1±0.8; isoproterenol, 2.4±1.6, n=10) and CAN×AC3-C mice (baseline, 2.5±1.0; isoproterenol, 3.1±2.1, n=10) than in CAN mice (Figure 1), perhaps because of a change in the genetic background of the interbred animals.

CaMKII is activated by increased stimulation frequency\(^14\) and isoproterenol,\(^4\) so we measured arrhythmias in Langendorff-perfused isolated hearts at baseline, during pacing, and after isoproterenol (Figure 7). There were no differences in arrhythmias at baseline, consistent with results in the ECG-telemetered mice. Pacing did not significantly affect arrhythmias in CAN×AC3-C hearts but reduced arrhythmias in CAN×AC3-I hearts, supporting the idea that CaMKII inhibition is antiarrhythmic during rapid stimulation. Isoproterenol caused a significant increase in arrhythmias compared with baseline in CAN×AC3-C hearts \( (P=0.003) \) but not in CAN×AC3-I hearts \( (P=0.85) \) and resulted in a trend \( (P=0.08) \) toward more arrhythmias in CAN×AC3-C compared with CAN×AC3-I hearts (Figure 7). These data suggest that arrhythmia suppression may contribute to reduced mortality in CAN×AC3-I compared with CAN×AC3-C mice (Figure 3F).

Discussion
CaMKII Significantly Determines Key Cardiomyopathy Phenotypes
The central finding from our studies is that CaMKII appears to be critical for determining clinically relevant disease...
Dephosphorylation of the nuclear factor for activated T cells is critical for the hypertrophic signaling action of CAN in heart, but CAN may affect other transcriptional signaling pathways, including myocyte enhancer factor 2 (MEF2). On the other hand, CaMK overexpression also activates MEF2 signaling to cause cardiac hypertrophy, whereas CAN is a comparatively less effective signal for MEF2 activation. Overall, our experiments do not support an important role for CaMKII in promoting cardiac hypertrophy initiated by CAN overexpression. However, our findings leave open the possibility that CaMKII inhibition may result in smaller and more abundant ventricular myocytes, perhaps by a developmental mechanism.

**CAN, CaMKII, and “Calcium-Dependent” Cardiomyopathy**

Disordered cellular Ca2+ handling is a consistent finding in patients and animal models of structural heart disease. Myocardial CAN overexpression was a seminal model for highlighting the concept that connections between altered cellular Ca2+ and Ca2+-activated cellular signaling molecules were important for determining clinically important cardiomyopathic phenotypes. Both CAN and CaMKII are pleiotropic molecules with multiple potential points for cross-talk, including cellular Ca2+ entry, cytoplasmic Ca2+ cycling through intracellular stores, and transcriptional signaling. The results of our study show that increased CaMKII activity and expression occur downstream of CAN activation and that CaMKII activity can link electrical and mechanical cardiomyopathic phenotypes. The role of CaMKII in directing cellular dysfunction and arrhythmias may explain why pharmacological CAN antagonists are ineffective in preventing arrhythmias, sudden death, and mechanical dysfunction in CAN-overexpressing mice. On the other hand, mice with TG expression of the myocyte-enriched CAN-interacting protein MCIP1 are protected from TG CAN expression and adverse remodeling after MI. The surprising role of CaMKII in CAN cardiomyopathy highlights the unanticipated complexity of “simple” monogenic models of structural heart disease.

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**Disclosures**

Dr Anderson is a named inventor on a patent to treat arrhythmias by CaMKII inhibition. The remaining authors report no conflicts.

**References**


Myocardial dysfunction and susceptibility to arrhythmias and sudden cardiac death are the most clinically important phenotypes in patients with structural heart disease. Improved understanding of how or whether these phenotypes are driven by specific signaling molecules may hold promise for developing drug therapies to address heart failure and arrhythmias. The multifunctional Ca\(^{2+}\) and calmodulin-dependent protein kinase II (CaMKII) has recently emerged as a candidate signal for causing cardiac dysfunction and arrhythmias in patients and animal models of structural heart disease. Findings reported here show that calcineurin increases CaMKII expression and that CaMKII inhibition by drugs or genetic approaches improves cardiac function, reduces arrhythmia susceptibility, and prolongs life in mice with calcineurin cardiomyopathy. These findings identify CaMKII as a “downstream” signal to calcineurin and suggest that inhibiting CaMKII may be effective for addressing clinical issues in advanced structural heart disease.

**CLINICAL PERSPECTIVE**


Khoo et al. CaMKII in Calcineurin Cardiomyopathy


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